Chromosomal Mapping of Canine-Derived BAC Clones to the Red Fox and American Mink Genomes

Anna V. Kukekova, Nadegda V. Vorobieva, Violetta R. Beklemisheva, Jennifer L. Johnson, Svetlana V. Temnykh, Dmitry V. Yudkin, Lyudmila N. Trut, Catherine Andre, Francis Galibert, Gustavo D. Aguirre, Gregory M. Acland, and Alexander S. Graphodatsky

From the James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Kukekova, Johnson, Temnykh, and Acland); the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia (Vorobieva, Beklemisheva, Yudkin, Trut, and Graphodatsky); Une unités mixtes de recherche 6061, Centre national de la recherche scientifique, Institute of Genetics and Development, Faculty of Medicine, Rennes, France (Andre and Galibert); and the School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Aguirre).

Address correspondence to Anna V. Kukekova at the address above, or e-mail: avk5@cornell.edu.

Abstract

High-quality sequencing of the dog (*Canis lupus familiaris*) genome has enabled enormous progress in genetic mapping of canine phenotypic variation. The red fox (*Vulpes vulpes*), another canid species, also exhibits a wide range of variation in coat color, morphology, and behavior. Although the fox genome has not yet been sequenced, canine genomic resources have been used to construct a meiotic linkage map of the red fox genome and begin genetic mapping in foxes. However, a more detailed gene-specific comparative map between the dog and fox genomes is required to establish gene order within homologous regions of dog and fox chromosomes and to refine breakpoints between homologous chromosomes of the 2 species. In the current study, we tested whether canine-derived gene–containing bacterial artificial chromosome (BAC) clones can be routinely used to build a gene-specific map of the red fox genome. Forty canine BAC clones were mapped to the red fox genome by fluorescence in situ hybridization (FISH). Each clone was uniquely assigned to a single fox chromosome, and the locations of 38 clones agreed with cytogenetic predictions. These results clearly demonstrate the utility of FISH mapping for construction of a whole-genome gene-specific map of the red fox. The further possibility of using canine BAC clones to map genes in the American mink (*Mustela vison*) genome was also explored. Much lower success was obtained for this more distantly related farm-bred species, although a few BAC clones were mapped to the predicted chromosomal locations.

Key words: Canis lupus familiaris, comparative genomics, FISH, Mustela vison, Vulpes vulpes

The phenotypic variation observed in domestic and farm-bred fur animals is striking, but its genetic basis is far from being well understood. New genetic tools and genome sequences (http://www.broad.mit.edu/mammals/) create an opportunity for the analysis of monogenic and complex phenotypes in dog, cat, and livestock species (reviewed in Georges 2007; Karlsson et al. 2007; Pontius et al. 2007; Wayne and Ostrander 2007). Furthermore, information from well-studied genomes can be applied for comparative analysis and genetic mapping of traits of interest in closely related species (de Gortari et al. 1998; Rogers et al. 2000, 2006; Reed et al. 2005; Anistoroaei et al. 2007). Here, we explore an opportunity to use canine genomic tools for assignment of gene-specific markers in the genomes of 2 farm-bred species, the red fox (*Vulpes vulpes*) and American

mink (*Mustela vison*), in which artificial selection has produced a variety of coat color, morphological, and behavioral phenotypes (Shackelford 1948, 1949; Belyaev et al. 1975; Robinson 1975; Nes et al. 1988; Våge et al. 1997; Trut 1999, 2001; Kharlamova et al. 2000, 2007; Trapezov et al. 2008).

Canids (dog and fox) and mustelids (mink) belong to the same Canoidea branch of the Carnivora and diverged from a common ancestor about 40 million years ago (Ma), whereas red fox and dog, members of the Canidae family, diverged about 10 Ma (Wayne et al. 1997; Bininda-Emonds et al. 1999). The dog karyotype (*Canis lupus familiaris*) comprises 37 pairs of acrocentric autosomes and a pair of sex chromosomes, whereas the red fox has 16 pairs of metacentric autosomes, a pair of sex chromosomes, and 0–8

Table 1. FISH localization of canine gene–containing BAC clones in the genomes of the silver fox and American mink

Gene name	BAC clone	Fox FISH mapping (VVU)	Mink FISH mapping (MVI)	CanFam2 canine ortholog location	Dog chromosome based on RH mapping data (CFA)	Closest RH markers	θ	LOD	Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)	Reference	Selected candidate genes	Genes that were previously mapped in the red fox by FISH
T-brachyury protein (*T* gene)	90-I8	1p1.5–2.6		Chr 1: 57, 228, 892-57, 237, 728	CFA1	FH3993	0.09	17.81	1164	AJ245513; Haworth et al. (2001)		
						FH3284	0.12	16.75	1164			
HTR1B	18-L8	1q3.5–3.6	1p26–27	Chr 12: 41, 662, 262-41, 662, 720	CFA12 (van den Berg et al. 2004)	BAC_372-C2	0.02	25.28	699	van den Berg et al. (2004)	n	
						EST5H8	0.02	25.28	694			
CRHR1	189-B1	2p2.10		Chr 9: 12, 791, 000-12, 841, 243	CFA9	BAC_372-C21	0.26	11.64	698	This study	n	
						CCL2	0.28	10.93	690			
GRIN2C	429-O8	2p2.10		Chr 9: 8, 582, 559-8, 594, 291	CFA9	GALK1	0.26	11.44		Sidjanin et al. (2003)		
						SRP68	0.37	8.09				
Tbx2 and Tbx4	327-P20	2p2.3–2.7		TBX2—Chr 9: 38, 559, 410-38, 567, 853	CFA9	FH2846	0.07	22.01	645	AY192796, AY192797; Andelfinger et al. (2003)		
				TBX4—Chr 9: 38, 487, 909-38, 513, 488		EST9A7	0.10	19.98	626			
SLC6A4	59-N4	2p1.5–2.5		Chr 9: 47, 549, 263-47, 572, 016	CFA9	EST27D2	0.03	19.82	820	This study	n	
						FH3235	0.12	16.90	811			
SLC6A3	89-K1	2p1.5-2.5		Chr 34: 14, 216, 551-14, 251, 928; Chr 2: 63, 201, 559-63, 242, 406 (CFA2 less likely)	CFA22	REN128H16	0.29	11.65	105	This study	n	
						AHTH211	0.35	10.04	128			
DBH	73-H9	2p1.5–2.5		Chr 9: 53, 342, 444-53, 361, 759	CFA9	LEI-2D2	0.19	11.80	967	This study	n	
						REN177B24	0.22	11.06	967			
C-KIT	118-O18	2p1.1		Chr 13: 50, 040, 774-50, 124, 361	CFA13	REN126A23	0.07	16.21	704	This study	c	Graphodatsky et al. (2005)
						KDR	0.08	15.42	711			

Table 1. Continued

Gene name	BAC clone	Fox FISH mapping (VVU)	Mink FISH mapping (MVI)	CanFam2 canine ortholog location	Dog chromosome based on RH mapping data (CFA)	Closest RH markers	θ	LOD	Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)	Reference	Selected candidate genes	Genes that were previously mapped in the red fox by FISH
KDR	67-P13	2p1.2		Chr 13: 50, 374, 957-50, 416, 670	CFA13 (Breen et al. 2004)					Graphodatsky et al. (2005)		Graphodatsky et al. (2005)
PDGFRA	311-A23	2p1.2		Chr 13: 49, 684, 632-49, 705, 871	CFA13	REN126A23	0.07	17.04	704	Graphodatsky et al. (2005)	c	Graphodatsky et al. (2005)
						C13.365	0.07	16.27	692			
SLC6A2	289-M11	2q3.4–3.7		Chr 2: 63, 198, 411-63, 242, 614	CFA2	FH2608	0.03	19.16	841	AC129099	n	
						BAC_372-A19	0.07	16.98	841			
GAD1	130-I2	3p2.2–2.6		Chr 36: 18, 369, 306-18, 412, 283	CFA36	EST19A10	0.08	23.79	348	This study	n	
						BAC_381-A3	0.17	18.82	330			
CYP3A26 (CYP3A4)	168-J21	3q1.5		Chr 6: 12, 784, 228-12, 821, 910	CFA6	BAC_382-E21	0.21	16.02	308	AC131027		
						BAC_372-O10	0.22	15.53	317			
ABAT	65-N8	3q2.1–3.1		Chr 6: 36, 426, 512-36, 474, 610	CFA6	REN88M24	0.09	13.65	709	This study	n	
						REN84K01	0.09	12.81	689			
CDH23	99-C20	4q1.6–2.2		Chr 4: 25, 492, 895-25, 781, 226	CFA4 (Kuiper et al. 2002)	BAC_99-C20	0.31	9.73	552	Kuiper et al. (2002)		
						BAC_381-H2	0.35	8.71	532			
DRD1	1-M13	4q1.3–1.7		Chr 4: 40, 743, 436-40, 746, 624	CFA4	FH3106	0.04	18.39	874	This study	n	
						FH2412	0.11	15.16	880			
ATOX1 pseudogene	84-B18	4q2.1–2.2	1q31–33	Chr 4: 60, 803, 406-60, 812, 281	CFA4	BAC_374-E21	0.05	14.10	1547	AF286877; van de Sluis et al. (2001)		
				Plus CFA18, 19, 20, and 24		FH1015	0.05	14.10	1547			
GABRG2	49-K24	4q3.1–3.3		Chr 4: 51, 998, 352-52, 105, 402	CFA4	EST11E12	0.08	15.48	1449	This study	n	
						REN282C02	0.11	14.39	1476			
HTR2A	127-D1	6p2.2–2.5		Chr 22: 7, 395, 321-7, 452, 531	CFA22	REN42F10	0.06	26.49	114	This study	n	Klukowska-Rötzler et al. (2005)
						REN128H16	0.04	26.25	105			

Table I. Continued

Gene name	BAC clone	Fox FISH mapping (VVU)	Mink FISH mapping (MVI)	CanFam2 canine ortholog location	Dog chromosome based on RH mapping data (CFA)	Closest RH markers	θ	LOD	Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)	Reference	Selected candidate genes	Genes that were previously mapped in the red fox by FISH
TYRP2 (DCT)	65-H10	6p1.3–1.4		Chr 22: 48, 220, 073-48, 253, 910	CFA22	DCT	0.03	20.52	592	This study	c	
						FH2662	0.09	17.97	578			
HOXA	78-A3	7p2.3								Li et al. (1999)		
GNGT1		7q1.1–1.3	3q14	Chr 14: 22, 363, 018-22, 367, 549	CFA14	BAC_372-I9	0.06	20.30	450	This study		
						BAC_375-O13	0.06	20.03	494			
SHARP1 (BHLHB3)	115-A9	8p1.2–2.1		Chr 27: 24, 475, 788-24, 480, 605	CFA27	EST7A1	0.28	9.64	394	Kukekova et al. (2003)		
						PTHLH	0.34	7.73	379			
ST8SIA1	333-F7	8p1.1–1.2		Chr 27: 27, 948, 915-28, 038, 424	CFA27	STS156J06	0.12	18.72	492	This study		
						D04813	0.14	17.65	502			
UGT1A6	252-I15	9p2.3–2.5		Chr 25: 48, 138, 801-48, 183, 812	CFA25	ALPI	0.06	18.49	835	AC093453		
						FH4027	0.07	17.64	844			
SLC6A1	102-D19	9q1.2–p1.1		Chr 20: 10, 344, 464-10, 386, 539	CFA20	REN55P21	0.21	17.12	226	This study	n	
						EST13G6	0.22	16.39	263			
IGF1	126-J3	10q2.1–2.2		Chr 15: 44, 229, 347-44, 285, 955	CFA15	FH2295	0.09	17.74	795	This study	m	Szczerbal et al. (2003)
						IGF1	0.14	15.70	808			
SLC25A3	50-E1	10q1–2.3		Chr 15: 41, 002, 937-41, 009, 210	CFA15	REN134K05	0.09	21.03	736	Debenham et al. (2001)		
						REN143N23	0.10	18.76	711			
TPH1	147-H22	11p2.3–2.4		Chr 21: 43, 739, 337-43, 757, 824	CFA21	SAA2	0.13	19.56	825	This study	n	
						EST11A11	0.14	18.58	783			
TECTA	59-C2	12q2.1–2.5		Chr 5: 15, 886, 369-15, 954, 803	CFA5 (Drögemüller et al. 2002)	REN42N13	0.06	19.67	385	Drögemüller et al. (2002)		
						REN78M01	0.06	19.17	397			
MYO15A	362-O13	12q2.9	8p11	Chr 5: 44, 369, 188-44, 426, 838	CFA5 (Rak et al. 2002)	BAC_375-M3	0.05	13.05	933	Rak et al. (2002)		

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Table 1. Continued

Gene name	BAC clone	Fox FISH mapping (VVU)	Mink FISH mapping (MVI)	CanFam2 canine ortholog location	Dog chromosome based on RH mapping data (CFA)	Closest RH markers	θ	LOD	Marker location on canine FISH-RH–integrated map, cR (Breen et al. 2004)	Reference	Selected candidate genes	Genes that were previously mapped in the red fox by FISH
MC1R	130-L5	12q3.1–3.3		Chr 5: 66, 692, 398–66, 693, 344	CFA5	H006	0.15	10.98	933	This study	c	
						MC1R	0.07	16.21	1421			
CNGB3	100-O2	13q1.1		Chr 29: 35, 752, 880-35, 896, 148	CFA29	K315	0.15	13.45	1433			
						FH2385	0.03	22.89	725	BH760135, BH760136; Sidjanin et al. (2002)		
LHX4	398-N19	13q4.4–4.6		Chr 7: 16, 740, 366-16, 782, 002	CFA7	FH2772	0.05	21.39	725	van Oost et al. (2002)		
						VIASD10	0.07	17.57	444			
ASIP	19-I2	14p1.4–1.5		Chr 24: 26, 327, 360-26, 366, 307	CFA24	CENPF	0.14	15.70	418	This study	c	
						REN95M13	0.14	16.21	653			
OTK and *AVP*	15-D10	14q1.1–p1.3		OTK—Chr 24: 21, 190, 516-21, 191, 062 AVP—Chr 24: 21, 180, 172-21, 181, 841		BAC_375-K22	0.15	15.10	686	This study	n	
PAX3	302-P11	16p1.1–1.4		Chr 37: 31, 348, 610-31, 446, 429	CFA37 (Lowe et al. 2003)					This study		
				Chr 2: 82, 718, 722-82, 809, 074								
MAOA	163-B12	Xp1.2–q1.1		Chr X: 37, 627, 026-37, 692, 688	X	REN172J16	0.06	19.83	334	This study	n	
Factor VIII (F8A)	292-C4	Xq2.3–2.4		Chr X: 125, 917, 495-126, 063, 641	X	FH2997	0.12	16.90	306	Lozier et al. (2002)		
						GDI1	0.12	9.92	1093			
						EST16E3	0.17	9.11	1112			

The location of the same genes in the CanFam2 canine genome assembly and on the canine RH map 5000 cR is indicated. "n," "c," and "m" indicate genes known to be involved in neurobiology and behavior, coat color, and morphology, respectively. BAC clone 15-D10 contains at least 2 genes OTK and AVP, and BAC clone 327-P20 contains genes Tbx2 and Tbx4. LOD, logarithm of the odds.

B chromosomes (Wipf and Scackeford 1942; Gustavsson 1964; Belyaev et al. 1974). The American mink's karyotype presents 14 pairs of bi-armed chromosomes plus a pair of sex chromosomes (Mandahl and Fredga 1975; Christensen et al. 1996). The cytogenetic relationships among dog, red fox, and American mink genomes is well understood, as fluorescence in situ hybridization (FISH) with whole-chromosome probes established the correspondence of chromosomal arms between the dog and fox, and the dog and mink karyotypes (Yang et al. 1999, 2000; Graphodatsky, Yang, O'Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2001, 2002).

Among the Canidae, the genome of the dog has been the most intensely studied to date. Genomic tools available for such studies include 2 bacterial artificial chromosome (BAC) libraries; meiotic, radiation hybrid (RH), and single nucleotide polymorphism maps; and an assembled genome sequence anchoring these resources (Werner et al. 1999; Breen et al. 2001, 2004; Kirkness et al. 2003; Hitte et al. 2005; Lindblad-Toh et al. 2005). The evolutionary closeness of the dog and fox provides an opportunity to apply canine genomic tools to study the fox genome. Recently, a meiotic linkage map of the silver fox (a coat color variant of the red fox) was constructed using dog-derived microsatellite markers (Kukekova et al. 2004, 2007). Alignment of the fox map against the dog genome identified highly conserved marker order between orthologous regions of dog and fox chromosomes.

Although some gene-specific markers have been placed on the fox meiotic linkage map, this approach has several limitations. Finding gene location by linkage mapping requires identification of gene-specific polymorphic markers and genotyping of these markers in reference 3-generation pedigrees. Furthermore, because alignment of the fox meiotic linkage map against the dog genome has identified pericentromeric regions of recombination suppression in fox chromosomes, marker order in these regions cannot be resolved unambiguously by meiotic linkage mapping.

Cross-species FISH mapping of BAC clones has been used previously for comparative mapping (Di Meo et al. 2005; Larkin et al. 2006; Davis et al. 2008), and several canine BAC clones have been mapped in the genome of the red fox and other canid species (Szczerbal et al. 2003, 2006; Graphodatsky et al. 2005; Klukowska-Rötzler et al. 2005). Two canine genome FISH maps are available, both anchored to the dog sequence assembly, with resolutions of 2 and 10 Mb, respectively (Thomas et al. 2005, 2007). A comparable FISH map of the fox genome would allow alignment to the dog genome at a high order of resolution than previously possible and provide a useful tool for genetic studies in foxes.

In the present study, 40 gene-containing canine BAC clones were selected to test whether such clones can be routinely used for gene mapping in foxes and were further evaluated to explore whether canine genomic tools might also be used to assign gene-specific markers in the genome of the American mink (*M. vison*), a more distantly related farm-bred species. The clones included 21 containing genes implicated in coat color, neurobiology, and skeletal development and selected as potential candidate genes for phenotypes segregating in these species.

Materials and Methods

Selection of Canine BAC Clones for Comparative Mapping

Forty clones (Table 1) were selected from the RPCI-81 canine 8.1-fold BAC library (http://www.chori.org/bacpac/mcanine81.htm). Nineteen of the selected clones had been previously identified as containing specific genes (Table 1), and a further 21 clones, each positive to a single gene-specific probe, were identified specifically for this study (Table 1) by screening the library as previously described (Kukekova et al. 2003). BAC DNA was extracted by alkaline lysis (Birnboim and Doly 1979). Polymerase chain reaction (PCR) amplification of BAC DNA with gene-specific primers (Supplementary Table 1) and sequencing were used to confirm that each selected clone contained the correct corresponding gene.

Slide and Probe Preparation

Metaphase chromosomal spreads were prepared from primary fibroblast and peripheral blood lymphocyte cultures of red fox (*V. vulpes*) and American mink (*M. vison*) as described previously (Yang et al. 1999; Graphodatsky, Yang, O'Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2002; Yudkin et al. 2007).

FISH Mapping

FISH on chromosomal spreads of red fox and American mink was performed using a standard protocol (Yang et al. 1999; Graphodatsky, Yang, O'Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000). Canine BAC clones were labeled by NICK Translation Kit (Invitrogen, Carlsbad, CA).

RH Mapping of Selected Genes on Canine 5000-centiray Panel

The 5000-centiray (cR) canine RH panel (Priat et al. 1998) was used for RH mapping of selected genes on dog chromosomes. Gene-specific primers were designed using sequences retrieved from the Trace Archive database (Supplementary Table 1). Primers for each gene were tested first on the DNA of dog and hamster, and only primer pairs that amplified the expected size product from canine but not hamster DNA were used for RH mapping. Each marker was amplified on the RH panel under the following conditions: 96 °C for 2 min; 30 cycles of 96 °C (20 s), 58 °C (20 s), and 72 °C (20 s); and a final extension at 72 °C for 5 min. All PCRs were performed using 25 ng of genomic DNA from each cell line in a final volume 15 μl, and the products were separated on a 1.8% agarose gel. PCR products were visualized by ethidium bromide staining. All markers were scored, and the complete marker set was analyzed together with 3270 markers previously mapped at
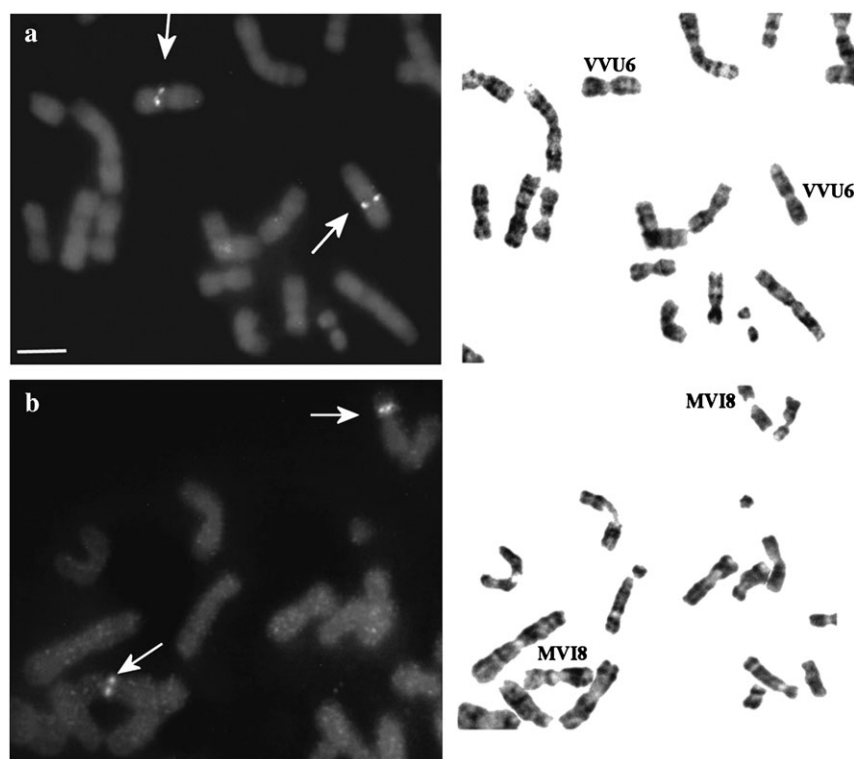
Figure 1. Examples of FISH mapping (left) of canine-derived BAC clones onto G-banded (right) metaphases of the red fox (VVU) and American mink (MVI). Localization of the TYRP2 onto VVU6p1.3–1.4 (**a**) and MYO15A onto MVI8p12–13 (**b**). Scale bar—10 μm.

the 5000-rad panel (http://www-recomgen.univ-rennes1.fr/Dogs/; Breen et al. 2004) using MultiMap (Matise et al. 1994). Two-point linkage analysis was performed for each selected gene to determine its nearest markers and chromosomal location. Presence of gene-specific amplicons produced for RH mapping in the corresponding canine BAC clones was confirmed by PCR.

Localization of Selected Genes in CanFam2

To identify positions of selected genes in the 7.6× sequence assembly of the dog genome (CanFam2), canine orthologs were located in the canine assembly by 1) gene names and 2) RH primer sequences mapped in silico using resources of the University of Southern California, Santa Cruz, Genome Bioinformatics Site (http://genome.ucsc.edu/).

Results

Localization of Selected Genes in the Dog Genome

RH Mapping

Two-point linkage analysis of 38 canine genes with 3270 markers previously mapped on RH map 5000 cR identified the chromosomal location of 36 genes (Table 1). For each tested gene, the position of at least 5 RH markers that

showed linkage with the highest support was identified on the RH_{5000} map (Breen et al. 2004). Genes were assigned to a particular chromosome when all such markers mapped to the same chromosomal region. The chromosomal locations of the 2 closest markers for each mapped gene on the RH_{5000} map (Breen et al. 2004) are listed in the Table 1. RH-mapped genes were assigned to 19 dog chromosomes with 8 chromosomes containing more than one gene (Table 1). The gene *AVP* has not been mapped; all primer pairs designed for this gene produced similar size band on hamster DNA. Location of HOXA could not be determined unambiguously. Two genes (*PAX3* and *KDR*) have not been mapped in this study, but location of these genes on the RH_{5000} map has been identified previously (Lowe et al. 2003; Breen et al. 2004).

Localization of Selected Genes in CanFam2

The unique locations of 35 selected genes in the CanFam2 assembly were identified using gene name as a query (Table 1). Five genes (*SLC6A3*, *SLC25A3*, *ATOX1* pseudogene, *PAX3*, and *HOXA*) were assigned to more than one chromosome in CanFam2 (Table 1). Four of these genes (*SLC6A3*, *SLC25A3*, *ATOX1* pseudogene, and *PAX3*) were localized uniquely in CanFam2 using in silico PCR with primer pairs used for BAC screening or RH mapping. Location of HOXA could not be determined unambiguously.
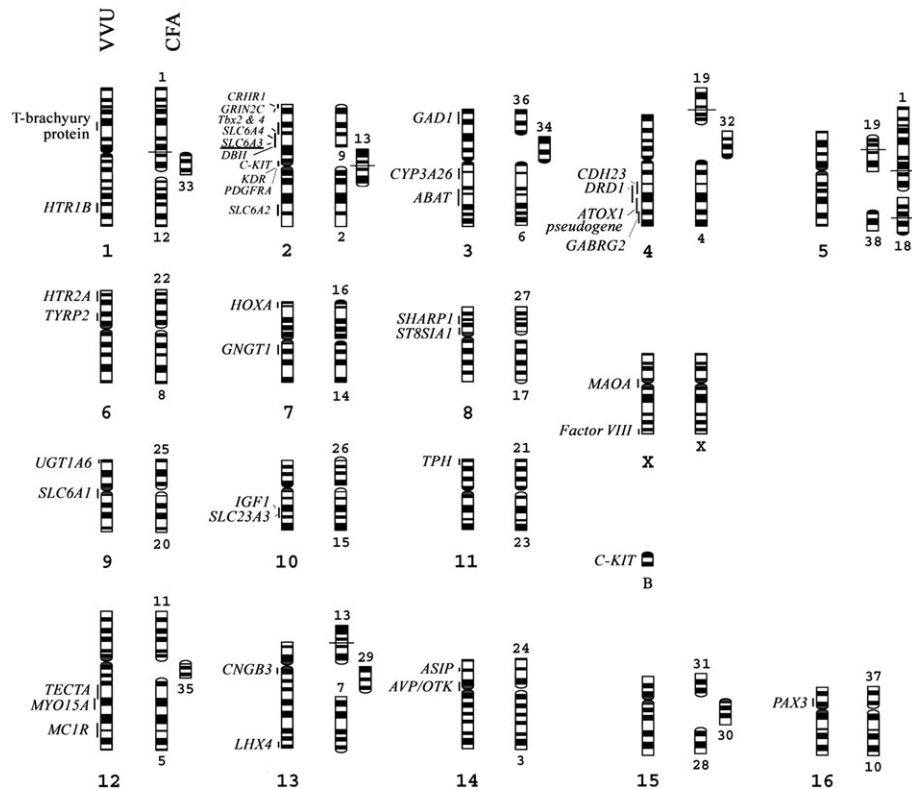
Figure 2. Chromosomal locations of 40 genes mapped in the fox genome by FISH. Positions of mapped genes are indicated on the comparative chromosomal map of the red fox and dog (modified from Yang et al. 1999, 2000) to simplify comparative analysis between 2 species. Dog chromosomes are labeled based on the chromosomal nomenclature used for canine genome sequence assembly (Breen et al. 1999; Lindblad-Toh et al. 2005). Fox chromosomes are located on the left side of each panel; dog chromosomes are on the right. Positions of the genes mapped in the fox genome by FISH are indicated on the left side of fox chromosomes. Each of dog chromosomes 1, 13, 18, and 19 are syntenic to 2 fox chromosomes. Horizontal bars drawn on dog chromosomes indicate breakpoints.

For 38 genes, the canine chromosomal locations on CanFam2 and the canine RH map were in agreement (Table 1). *SLC6A3* and *AVP* were the exceptions: *SLC6A3* was assigned to CFA22 by RH mapping but is most likely located on CFA34 in CanFam2, and *AVP* was identified on CanFam2 but not on the RH Map.

Localization of Canine BAC Clones to Fox Chromosomes by FISH

All 40 canine gene–containing BAC clones were uniquely mapped to fox chromosomes by FISH. BAC clones were assigned to 15 of 17 fox chromosomes (Table 1 and Figure 1a). More than one BAC clone were assigned to 13 fox chromosomes: Two BACs were assigned to VVU1, 6, 7, 8, 9, 10, 13, 14, and X; 3 BACs were assigned to VVU3 and 12; 4 BACs were assigned to VVU4; and 10 BACs were assigned to VVU2 (Table 1 and Figure 2).

Comparative locations of 38 genes on the fox and dog chromosomes were in agreement with comparative cytogenetic data. Localization of a BAC clone containing *SLC6A3* gene on VVU2 was in disagreement with both the most

likely location of this gene in CanFam2 (CFA34) and its location predicted by RH mapping (CFA22). Because *HOXA* has not been identified in the dog genome, we could not make a comparison between dog and fox for this gene. The BAC containing the gene *C-KIT* localized to both VVU2 and B chromosomes as described previously for other *C-KIT* BAC clones (Graphodatsky et al. 2005).

These newly located genes, together with those previously mapped in the fox genome using hybrid cell lines (Rubtsov et al. 1988, 1998), increased the total number of gene-specific markers in the fox genome to 77 (Supplementary Table 2).

Localization of Canine BACs to Mink Chromosomes

Four BAC clones, containing the genes *HTR1B*, *GNGT1*, and *MYO15A* and the *ATOX1* pseudogene, were uniquely assigned to mink chromosomes (Figures 1b and 3) and in agreement with comparative cytogenetic data for mink and dog genomes (Figure 3 and Table 1). In each case, these 4 gene-containing BAC clones identified segments of mink chromosomes that were orthologous to the corresponding fox chromosomes (Figure 2, Table 1).
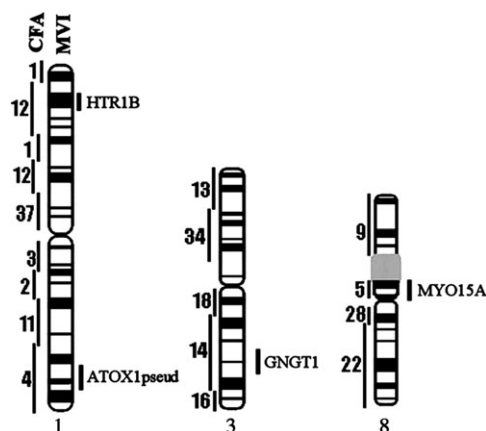
Figure 3. Subchromosomal localization of 4 canine BAC clones onto American mink chromosomes. Homology of American mink and dog chromosomes has been described previously (Graphodatsky, Yang, O'Brien et al. 2000; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2002). Numbers beside the vertical lines (left) correspond to dog chromosomes. Vertical black bars on the right side of each fox chromosome show location of canine BAC clones (this study).

Discussion

Forty canine-derived BAC clones containing known genes were uniquely assigned to fox chromosomes by FISH. Among selected BAC clones, 15 contain genes implicated in neurobiology and behavior, 5 clones contain genes involved in coat color in mammals, and at least 1 clone contains a gene important for morphology. Selection of these genes for FISH mapping in the fox genome was predetermined by the needs of ongoing genetic studies in foxes. The rest of the clones used in this study represent previously characterized BAC clones selected to increase the number of probes.

When BAC clones for FISH mapping were selected for this study, the RPCI-81 BAC library was the only available well-characterized canine BAC library. In contrast to the canine library CHORI-82 that was subsequently used for the dog genome sequencing (Lindblad-Toh et al. 2005), RPCI-81 clones are not anchored in the canine sequence assembly, and the location of each clone in the dog genome had to be identified de novo. To do so, both in silico localization of these genes in CanFam2 and RH mapping were used. The chromosomal localization of 36 of these genes in the dog was in agreement for the 2 methods. Location of 2 genes was in disagreement (Table 1). The *HOXA* gene was not uniquely localized in the dog genome by either method. The BAC clone containing *HOXA* gene had been identified by screening the RPCI-81 BAC library with the probe AF103746 (National Center for Biotechnology Information database; Li et al. 1999). This probe is 118 bp long and most likely does correspond to the *HOXA4* gene on CFA14, although the statistical support for this location is not significantly higher than for several other *HOXA* genes. RH mapping with primers based on AF103746 did not identify

a unique location for this gene in the dog genome either. For this reason, this gene was excluded from the comparative analysis of the dog and fox.

Comparison of gene locations between the fox and dog genomes was performed to test whether gene locations identified in the fox genome by FISH agreed with their chromosomal locations predicted from comparative cytogenetic data and alignment of the fox meiotic linkage map to the dog genome. The locations of 38 genes mapped in the fox genome agreed with these predictions. Location of one gene, *SLC6A3*, was in disagreement. The most likely location of this gene in CanFam2 is on CFA34, which is homologous to fox chromosome 3, but the BAC 89-K1 carrying *SLC6A3* gene was mapped to the fox chromosome 2, close to the *SLC6A4* gene. Similarity between the coding regions of these 2 genes or chimeric structure of the BAC 89-K1 can contribute to unpredicted localization of this gene. Alternatively, but less likely, the *SLC6A4* mapping results might also indicate a small insertion in VVU2 corresponding to part of CFA34 that has not yet been identified.

Previously, FISH localization of 3 canine BAC clones in 4 canids—the dog, red fox, arctic fox, and Chinese raccoon dog—has been reported (Szczerbal et al. 2003; Klukowska-Rötzler et al. 2005). These clones were selected from a different dog BAC library (http://www.dogmap.ch/getclone.html, Schelling et al. 2002), but 2 of them contain genes included in the current study (*IGF1* and *HTR2A*). Our results confirmed localization of the *IGF1* gene onto VVU10q chromosome and *HTR2A* on VVU6p.

In the current study, 2 genes (*TYRP2* and *HTR2A*) were mapped to the short arm of fox chromosome 6, which is homologous to CFA22. *TYRP2* mapped close to the centromere, whereas *HTR2A* mapped close to the telomere. According to the CanFam2 annotation, the location of these genes on CFA22 is opposite, *TYRP2* being near the dog telomere, whereas *HTR2A* is located close to the centromere. The relative positions of *HTR2A* and *TYRP2* genes on VVU6 indicate that the segment homologous to CFA22 has the opposite orientation in VVU6 relative to that reported by comparative chromosomal painting (Graphodatsky et al. 2001, Figure 2). This observation is also supported by previous FISH mapping results for *HTR2A* (Szczerbal et al. 2006) and by alignment of the fox meiotic linkage map against the dog genome (Kukekova et al. 2007).

Previously, more than 40 gene-specific markers have been localized in the fox genome using a panel of somatic cell hybrids (Rubtsov et al. 1988, 1998). These data, together with mapping data reported in Szczerbal et al. (2003, 2006), and FISH mapping results from the current study bring the number of genes mapped onto specific fox chromosomes to 77 (Supplementary Table 2).

In the current study, we found that FISH mapping with canine-derived BAC clones is an efficient approach for mapping genes in the silver fox genome, as all 40 canine BAC clones used in our study were uniquely assigned to fox chromosomes. Locations of 38 genes were in agreement with the cytogenetic predictions; locations of 2 genes were inconclusive. This is not unexpected as there is some

inconsistency even when canine BAC clones are FISH mapped in the dog (Breen et al. 2004). When an unpredicted location of a BAC clone is identified, additional BAC clones from the same genomic region should be mapped to understand whether observed results were caused by chimeric BAC structure, sequence similarity between different genes, or chromosomal insertions that were not detected previously. Results of this pilot project indicate an opportunity for large-scale mapping of the fox genome with canine-derived BAC clones. BAC clones from the CHORI-82 library are well characterized and anchored in the CanFam2 dog genome assembly (Lindblad-Toh et al. 2005). Mapping of the dog genome with panels of CHORI-82 BAC clones demonstrated that multicolor FISH allows construction of a gene map of the dog genome with the resolution up to 2 Mb (Thomas et al. 2005, 2007). A high-density gene map of the fox genome constructed using multicolor FISH would allow high-resolution comparison of the chromosomal regions between fox, dog, and human, refining breakpoints between homologous chromosomes, characterization of the telomeric ends of fox chromosomes, and establishing gene order between homologous regions of dog and fox chromosomes. A fox–dog comparative gene map will be an important resource for genetic studies in foxes, allowing localization of fox-mapped loci in the dog genome, selection of canine microsatellite markers for fine mapping of these loci in foxes, and identification of positional candidate genes. Furthermore, construction of high-resolution comparative FISH maps for different canid species will provide new insights into the karyotypic evolution of the Canidae.

FISH mapping results for canine-derived BAC clones in the American mink were less successful. Only 4 canine BAC clones were uniquely assigned to mink chromosomes (Figure 3, Table 1). Previous reports of FISH mapping of bovine-derived BAC clones in the genome of American mink were more encouraging (Larkin et al. 2006). These different success rates may represent differences in protocol or gene selection. A BAC library of the European ferret (*Mustela furo*) that has recently become available (https://bacpac.chori.org/library.php?id=330), together with the first meiotic linkage map of the American mink (Anistoroaei et al. 2007), should provide valuable resources for genetic studies in *Mustelida*. Locations of the 4 genes mapped by FISH in mink were in agreement with cytogenetic predictions based on the established homology between the dog and mink chromosomes (Yang et al. 1999; Graphodatsky, Yang, Serdukova et al. 2000; Graphodatsky et al. 2002). The fact that these 4 probes identified regions of the mink and the fox genomes, which were homologous to the same dog chromosomes, demonstrates that there is utility for this method of comparative mapping and gene identification in the 3 species.

Supplementary Material

Supplementary Tables 1 and 2 can be found at http://www.jhered.oxfordjournals.org/.

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